

Electronic Supplementary Information for:

Bimetal Metal-Organic Framework Domino Micro-Reactor for Synergistic Antibacterial Starvation/Chemodynamic Therapy and Robust Wound Healing

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1. Experimental

1.1 Preparation of Bimetal MOF and BMOF-DMR

Zn(NO₃)₂·6H₂O (2.4 mmol, Chengdu Chron Chemicals) and Cu(NO₃)₂·3H₂O (1.6 mmol, Chengdu Chron Chemicals) were dissolved in 40 mL of deionized (D.I.) water. Next, 150 mL of 2-methylimidazole aqueous solution (1 M, Chengdu Chron Chemicals) was mixed with the above solution, and reacted with Zn²⁺ and Cu²⁺ ions for 24 h to form bimetal MOFs. The attained products (Cu/Zn bimetal MOF) were centrifuged at 10,000 rpm for 30 min, followed by washing with D.I. water at least three times and drying at 60°C. Afterward, GOx-laden bimetal MOFs (i.e., BMOF-DMR) were synthesized via immersing 10 mg of bimetal MOFs into a 2 mg/mL GOx solution (100 μL, Aladdin, China) and drying at 4°C for subsequent characterization and further use.

1.2 Sample Characterization

Surface morphology was detected using a scanning electron microscope (SEM, JSM-7500-F, JEOL, Japan) and transmission electron microscope (TEM, Tecnai G2 F20 S-TWIN, FEI, USA). The element composition and distribution of the as-prepared BMOFs were investigated by an energy dispersive X-ray accessory equipped on TEM. An X-ray diffractometer (XRD, Xcalibur A Ultra, Oxford, UK) was employed to examine the phase composition of samples at the Cu target radiation ($\lambda = 1.5444 \text{ \AA}$) with a measuring range of 10°-90°. A Fourier transform infrared spectrometer (FTIR, Nicolet 6700, TFS, USA) was used to investigate the functional groups in samples. X-ray photoelectron spectroscopy (XPS) was utilized to explore the composition and valence state of elements of samples. Brunauer-Emmett-Teller (BET) spectra were recorded by an automated physics sorption analyzer (APSA, Mike2460, MICROMERITICS, USA). Zeta potential and hydrodynamic diameter measurements were carried out by a Zeta potential analyzer (Zetasizer Nano ZS, Malvern, USA). Electron spin resonance (ESR, JES-FA200, JEOL, Japan) assays were performed using 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) as the spin-trapping agent for ·OH radicals.

1.3 Colloidal Stability

The colloidal stability of samples was measured to evaluate the dispersion and stability under liquid condition. Briefly, 4 mL of samples (1 mg/mL) were placed in the little glass bottle and let set for two days. At the predetermined periods (0, 8, 24, 48 h), the Tyndall effect of the solutions were tested using a red laser.

1.4 Copper Content Evaluation

Cu ions content in BMOF-DMR was detected by an inductively coupled plasma mass spectrometry (ICP-MS, VG PQExCell, TJA, USA). Briefly, 10 mg of BMOF-DMR was immersed in 50 mL of HCl solution (1M, pH=0, 37°C) for 3 days to ensure the completely release of Cu²⁺. Then, the content of Cu²⁺ in BMOF-DMR was detected by the ICP-MS.

1.5 GOx Content Detection

Since GOx is the only protein in BMOF-DMR, the protein content can represent the GOx content in BMOF-DMR. The bicinchoninic acid (BCA) is a stable water-soluble compound, and bivalent Cu²⁺ can be reduced into Cu⁺ in alkaline conditions by proteins. Cu⁺ can interact with BCA and form a purple complex and the compound is water-soluble, which has an absorption peak at 562 nm. The protein content in BMOF-DMR (10 µg/µL, 20 µL) was measured by the BCA detection kits (KeyGEN, Jiangsu) following the manufacturer's instructions. The photographs of the final solutions were recorded by the digital camera.

1.6 Detection of Ion Release

The delivery of Zn and Cu ions from BMOF-DMR in PBS solution was recorded. Briefly, 10 mg of BMOF-DMR was placed in a dialysis bag and immersed in 40 mL of PBS (pH = 7.4, 37°C). The system was continuously shaken at a speed of 100 rpm at 37°C. At predesigned intervals, 5 mL of release medium was collected for measurement and refilled with fresh medium at 37°C. The amount of Zn and Cu ions delivered from BMOF-DMR was detected through the ICP-MS. To assess the biodegradability of the products, BMOF-DMR was soaked in neutral and acidic buffers (pH = 7.4, 5.6, and 3.8) at 37°C for 7 days (d), and its morphology was characterized through SEM.

1.7 GSH Consumption

The depletion of GSH, which is the main endogenous antioxidant in bacteria, was measured through the colorimetric method. A total of 450 µL of GSH (1 mM) was added into 50 µL of sample dispersions (100, 200, 300, 400, 500 µg/mL) and incubated for 1 h. After that, samples were submerged into 450 µL of GSH reaction solution (1

mM in carbonate buffer, pH = 9.6) and incubated in the dark for 1 h. All solutions were collected and centrifuged to remove the samples. Then, the supernatant was mixed with 450 μ L of Tris-HCl (50 mM, pH = 8.0) and 100 μ L of 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB, 10 mM) and shaken for 30 min. H₂O₂-treated GSH solution served as the positive control, and the unsettled GSH solution served as the negative control. The color alteration of the hybrid solutions was recorded by a digital camera (D7100, NIKON, Japan), and the absorbance of the mixed solutions was measured from 250 nm to 500 nm *via* a UV-vis-NIR spectrophotometer (UV-3600, Shimadzu, Japan). Quantitative results were obtained by measuring the optical density of the solutions at 410 nm and the following formula:

$$\text{Loss of glutathione (\%)} = (Nc-S) / (Nc-Pc) \times 100 \%, \quad (1)$$

where *Nc* represents the OD value of the negative control, *Pc* represents the positive control, and *S* indicates the OD value of the solution.

1.8 Detection of H₂O₂ Generation

The detection of H₂O₂ generation was carried out based on the TMB-H₂O₂ reaction. In detail, 100 μ L samples or GOx were mixed with 900 μ L of TMB (3 mM, dissolved in acetate buffer). Then, 100 μ L of glucose (5 mg/mL) was incorporated into the mixture and incubated for 2 h in the dark. The absorbance of the supernatant was measured at 300-800 nm using a UV-vis-NIR spectrophotometer, and the photographs were obtained through the digital camera.

The quantitative content of generated H₂O₂ was measured through the H₂O₂ detection Kits (KeyGEN, Jiangsu). In brief, 50 μ L of samples (400 μ g/mL) were mixed with 450 μ L of glucose (5 mg/mL) and incubated for 30 minutes. Then, the supernates were collected for H₂O₂ content detection following the manufacturer's instructions.

1.9 Detection of \cdot OH Generation

Methylene blue (MB) was utilized to detect the generation of \cdot OH. Specifically, 4.3 mL of MB (20 μ g/mL) and 0.7 mL of BMOF-DMR (500 μ g/mL) mixed aqueous solution was kept in the dark for 30 min to satisfy an absorbance-desorption equilibrium. Afterward, 1 mL of glucose and 1 mL of H₂O₂ (10 mM) were added to the reaction system and incubated at 37 °C. The absorbance of the mixed solution was recorded at 550-700 nm using a UV-vis-NIR spectrophotometer, and the photos of diluent solutions (1×10^3) were taken by the digital camera.

1.10 *In Vitro* Antibacterial Assays

1.10.1 Spread Plate Assay

The antibacterial effects of BMOF, BMOF-DMR, and GOx against Gram-negative *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*) bacteria were evaluated by the spread plate method. Briefly, 450 μL of the bacteria suspension (1×10^5 colony-forming units (CFU)/mL, containing 0.5 w/v % glucose) was incubated with the 50 μL samples at various concentrations (0, 10, 20, 30, 40, 50 $\mu\text{g}/\text{mL}$) at 37°C for 5 h. Afterward, 50 μL of the bacteria suspension was evenly inoculated and spread on solid agar plates. After 24-h incubation, photographs of the agar plates were obtained *via* the digital camera to quantify the antibacterial efficiency.

1.10.2 Antibacterial Kinetic Tests

Antibacterial kinetic tests were used to evaluate the sustained bactericidal activity of the samples. Briefly, 2 mL of bacteria suspension at a high density (1×10^7 CFU/mL) and samples were incubated for the predetermined periods (0, 0.5, 1, 2, 3, 4, 5 h), and the supernatant was collected to measure the absorbance at 600 nm by a microplate reader (SAF-6801, BAJIU).

1.10.3 Alteration of pH Value in Antibacterial assay

The alteration of pH value in antibacterial assay was explored to validate the formation of acid condition. Briefly, 450 μL of the bacteria suspension (1×10^5 CFU/mL, containing 0.5 w/v% glucose) was incubated with the 50 μL samples at 37°C for 5 h. And the pH values of each sample were detected by the pH test strips for every one hour.

1.10.4 Live/Dead Assay of Bacteria

LIVE/DEAD™ BacLight Bacterial Viability Kits (Thermo Fisher Scientific, China) were utilized to evaluate the bactericidal effect of the samples, where live (green) and dead (red) bacteria were stained by SYTO 9 and propidium iodide (PI), respectively. Briefly, 450 μL of bacteria (1×10^6 CFU/mL) was seeded onto a 48-well plate and pre-incubated for 4 h to allow for bacterial adhesion. Next, different samples were incorporated into the bacterial suspension and cultured for 5 h. Afterward, both *E. coli* and *S. aureus* treated by the different samples were stained with SYTO 9 and PI for 15 min. The fluorescence signals of bacteria were measured using a fluorescence microscope (CKX53, OLYMPUS, Japan).

1.10.5 Morphology Observation of Bacteria

The bacterial morphologies were examined by SEM after the antibacterial experiment. After removing redundant solution, the adherent bacteria were rinsed, fixed with 200 μL of glutaraldehyde (2.5 %) for 1 h, and dehydrated in gradient alcohol

(30 %, 50 %, 70 %, 80 %, 90 %, and 100 %). After drying, the bacterial morphology and integrity were observed using SEM.

1.11 *In Vitro* Cytocompatibility Assays

1.11.1 Cytotoxicity Assay

The mouse fibroblast L929 cell was supplied by the West China Hospital of Sichuan University. The cells were cultured with Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 10 % fetal bovine serum (FBS, KeyGEN, Jiangsu) and 1 % penicillin-streptomycin (KeyGEN). Briefly, L929 cells seeded onto 48-well plates (4×10^4 cells per well) were incubated with a series of concentrations ranging from 10 to 40 $\mu\text{g}/\text{mL}$ of BMOF and BMOF-DMR for 1, 3, or 5 d. Then, 300 μL of DMEM medium and 30 μL of CCK-8 were added to each well and incubated for another 3 h. The absorbance of 100 μL of supernatant from each sample was measured at 450 nm by a microplate reader.

1.11.2 Lived/Dead Cell Assay

The Live & Dead Viability/Cytotoxicity Assay Kit for Animal Cells (KeyGEN, Jiangsu) was used to stain cells incubated with BMOF-DMR for 1 d, in which live (green) and dead (red) cells were stained by Calcein-AM and PI, respectively. Briefly, 500 μL of L929 cells (4×10^4 cells per well) were seeded and incubated for 1 d. Afterward, the samples were mixed with the media containing cells and incubated for another day. Then, the supernatants were removed, and the wells were washed and stained with the prepared dyes. The fluorescence images of cells were measured through a fluorescence microscope.

1.11.3 Cell Morphology Staining

L929 cells (4×10^4 cells per well) were first incubated with various samples for 1 d. After removing the samples, the cells were fixed with formaldehyde (4 %) for 20 min and rinsed several times. Then, the fixed cells were subjected to permeabilization in 0.1 v/v % TritonX-100 in PBS for 20 min. Afterward, the cytoskeleton was stained by fluorescein isothiocyanate phalloidin (FITC-phalloidin, Solarbio, Beijing) for 30 min, followed by counter-staining of cell nuclei by 4', 6-diamidino-2-phenylindole (DAPI, Solarbio) for 1 min. After rinsing, the fluorescence signals of cells were captured by fluorescence microscopy.

1.11.4 Enzyme-Linked Immunosorbent Assay (ELISA)

To explore the capability of samples to promote angiogenesis, ELISAs were carried out to detect the expression of intracellular vascular endothelial growth factor (VEGF).

Briefly, L929 cells with 1×10^4 cells per well were seeded and incubated with samples for 3 d. After that, the samples were centrifuged and supernatants collected for the VEGF ELISA following the manufacturer's instructions.

1.12 Antibacterial Assay *In Vivo*

Female Balb/c mice (7 weeks) were purchased from Chengdu Dossy Experimental Animals Co., Ltd. All the animal care and experimental protocols were approved and complied with the instructions of Medical Ethics Committee of Sichuan University (No. 2021605A). To explore the antibacterial ability of samples *in vivo*, 6-8-week-old female Balb/c mice were randomly divided into four groups, with four animals per group: PBS, ZIF-8, BMOF, and BMOF-DMR. After 1 week of accommodation, a round, full-thickness cutaneous wound (diameter: 8 mm) was created on the dorsal sites of mice by surgical scissors and infected with 20 μ L of *S. aureus* solution (1×10^8 CFU/mL). The infected wound was sealed using 3M™ Tegaderm™ transparent film dressing (3M, USA) to ensure successful infection. After 1 d, the infected wounds were dropwise added with 30 μ L of PBS, ZIF-8, BMOF, or BMOF-DMR (40 μ g/mL). The interstitial fluid from the wounds was collected and cultured in 2 mL of Luria-Bertani (LB) media. The spread plate method and optical density measurement were performed using the interstitial fluid to investigate the antibacterial efficacy *in vivo*. The weights of mice and photos of wounds were also recorded daily. After 7 d post operation, the mice were humanely killed, and the skin wound tissues and major organs (heart, liver, spleen, lung, and kidney) were harvested for further histopathological analysis.

1.13 Histopathological Evaluation

The regenerating wound tissues were harvested using a scalpel and fixed in formalin. After dehydration in gradient ethanol solutions and infiltration with xylene, wound tissues were embedded in paraffin wax and sectioned (5 μ m) for further tissue analysis. Hematoxylin and eosin (H&E) and Masson trichrome staining were carried out to investigate the healing of wounds through an optical microscope (UB2031, UOP, Chongqing). Giemsa staining was acquired to assess the wound infection and surviving bacteria. For immunohistochemical analysis, CD34 and VEGF staining were performed using the corresponding antibodies (Proteintech, USA). Furthermore, the wound sections were stained with TNF- α to explore inflammation in the wounds. To evaluate the biosafety of samples *in vivo*, the major organs were sectioned and stained with H&E. All the blood samples of mice were analyzed by blood biochemistry.

1.14 Statistical Analysis

All data were shown as mean \pm standard deviation based on at least three tests and contrasted *via* Kruskal-Wallis one-way analysis of variance (ANOVA).

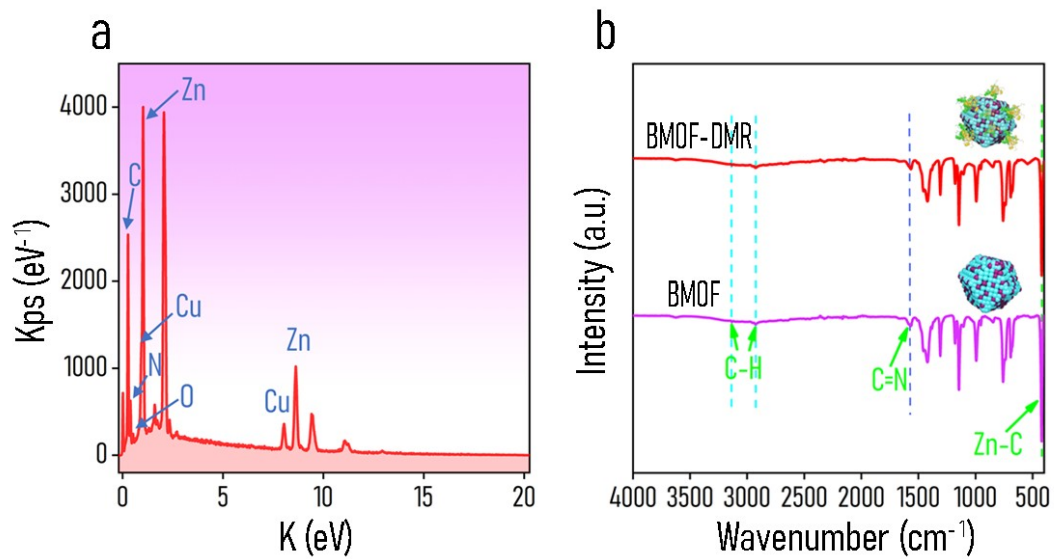


Figure S1. (a) EDS pattern of BMOF; (b) FT-IR spectra of BMOF and BMOF-DMR.

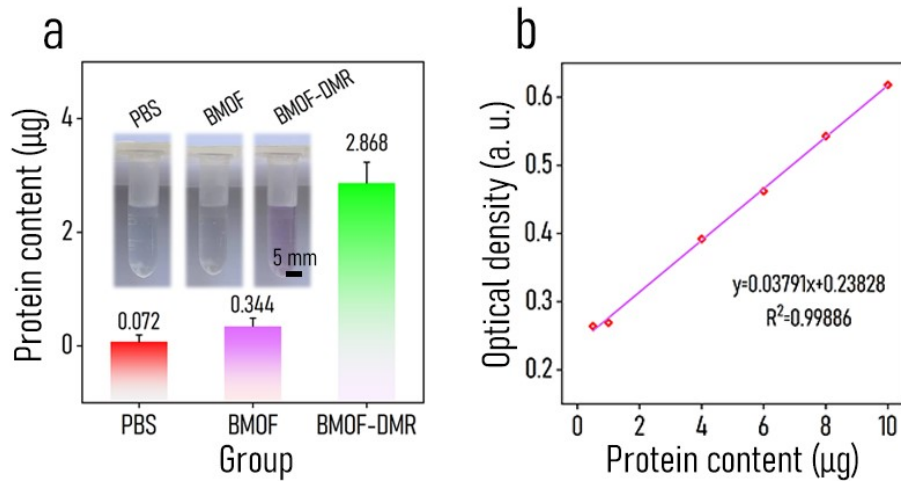


Figure S2. (a) GOx protein content of different samples and (b) standard curve of BCA detection kit.

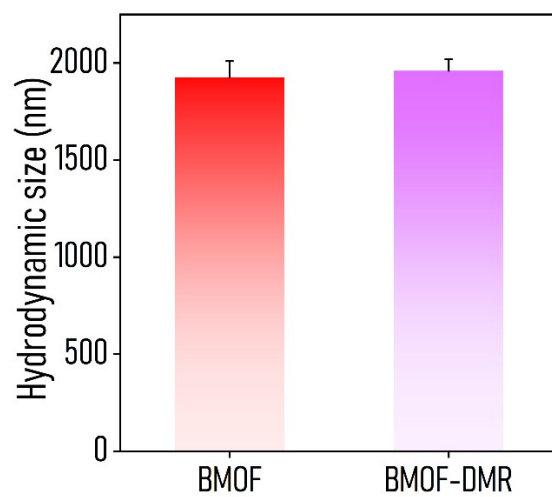


Figure S3. Hydrodynamic sizes of BMOF and BMOF-DMR. Data were presented as mean \pm s.d. (n=3).

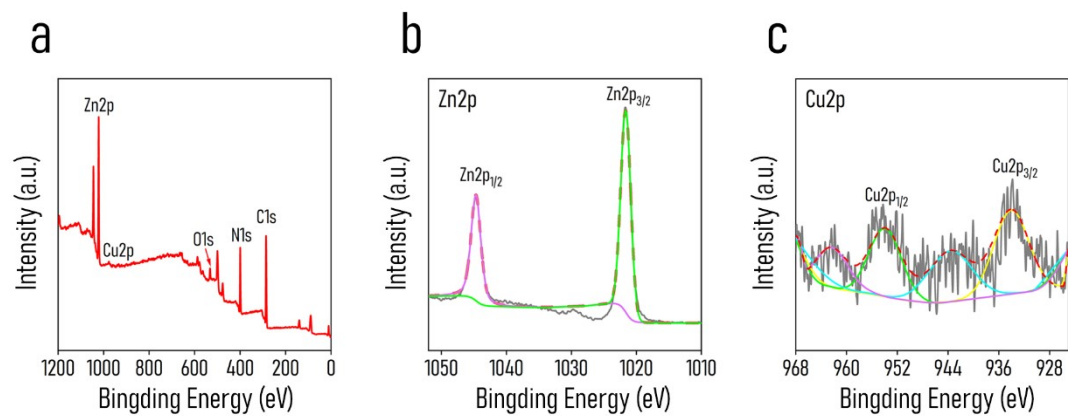


Figure S4. (a) Full-scan XPS survey of BMOF-DMR; High-resolution XPS spectra for (b) Zn2p and (c) Cu2p of BMOF-DMR.

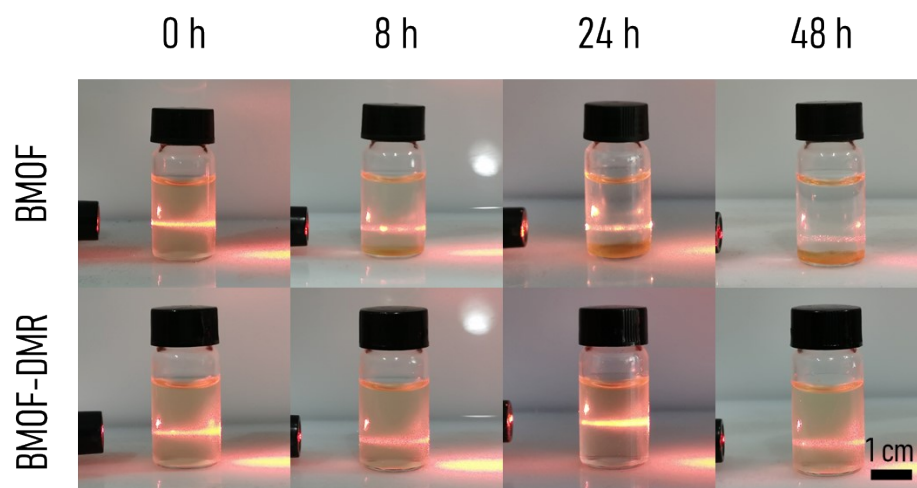


Figure S5. The images of samples' Tyndall effect after setting for different time.

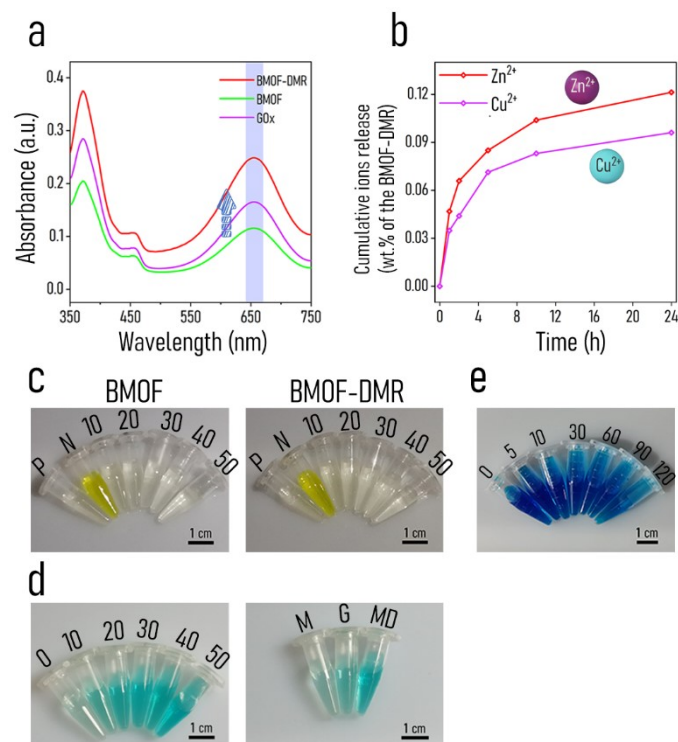


Figure S6. (a) UV-vis spectra of TMB reductions for different samples at 50 µg/mL; (b) Zn²⁺ and Cu²⁺ ions release curves of BMOF after immersion in PBS (37°C, pH 7.4) for 24 h; The corresponding color alteration images of (c) GSH, (d) TMB and (e) MB solutions treated with different samples and time. (Inserted in (c) and (d), P: positive control, N: negative control, 0-50: 0-50 µg/mL, M: BMOF, G: GOx, MD: BMOF-DMR; Inserted in (e), 0-120: 0-120 minutes.)

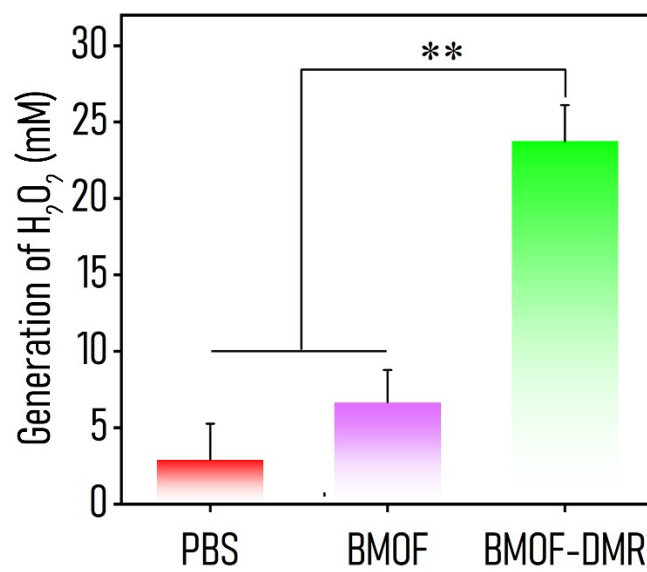


Figure S7. The generated H₂O₂ content of samples after 30 minutes' incubation with glucose solution.

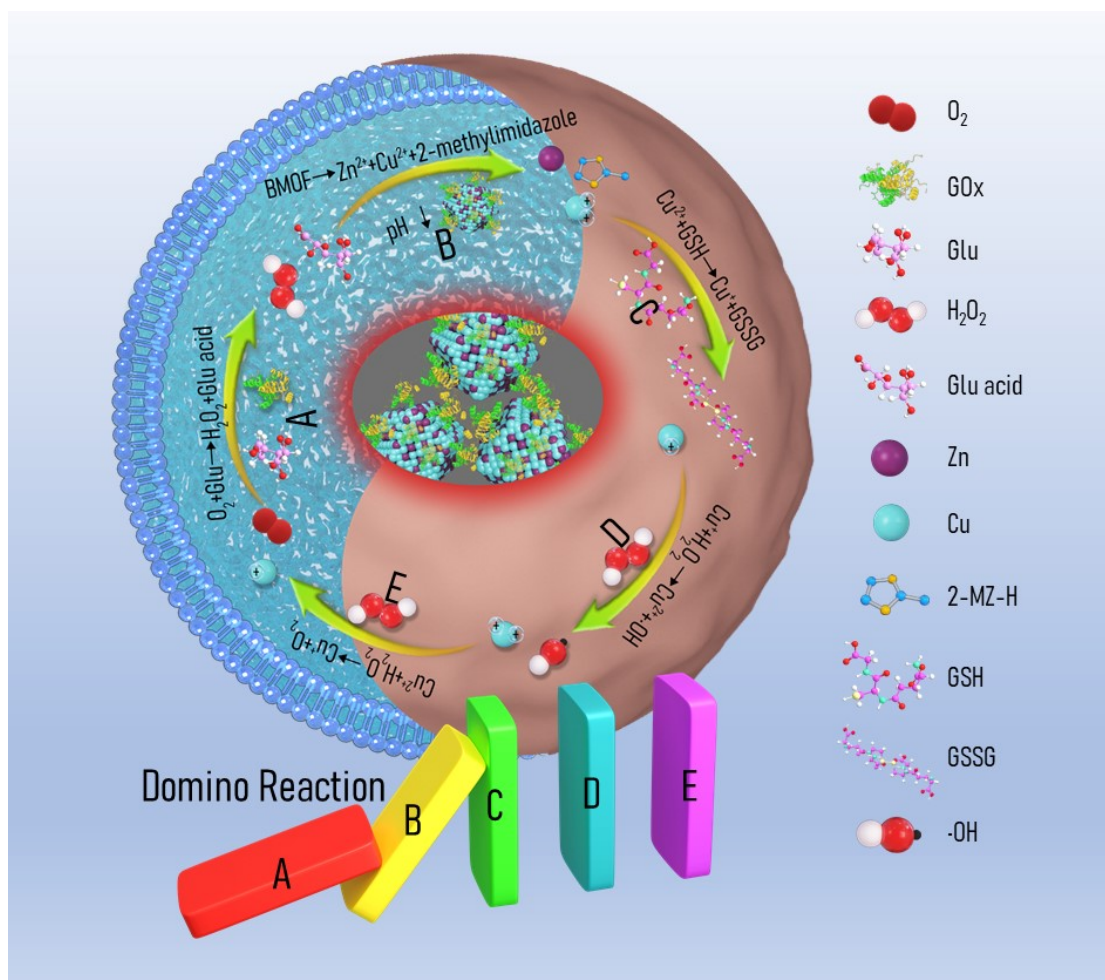


Figure S8. Scheme of domino catalytic reaction.

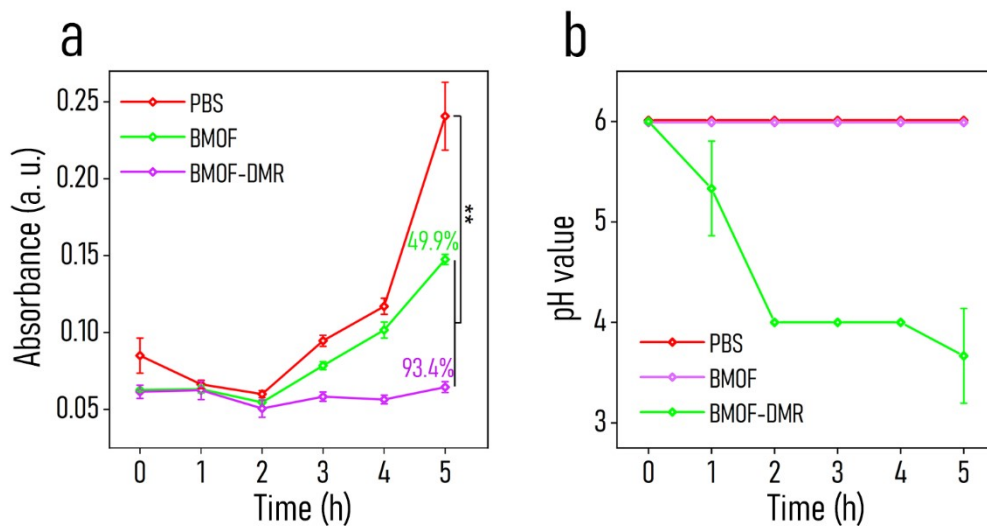


Figure S9. (a) Bacterial growth kinetics curves of *E. coli* treated with PBS, BMOF, and BMOF-DMR; (b) The alteration of pH value in antibacterial assay. (Percentages inserted in (a) show the calculative antibacterial ratio of BMOF and BMOF-DMR after incubation for 5 h).

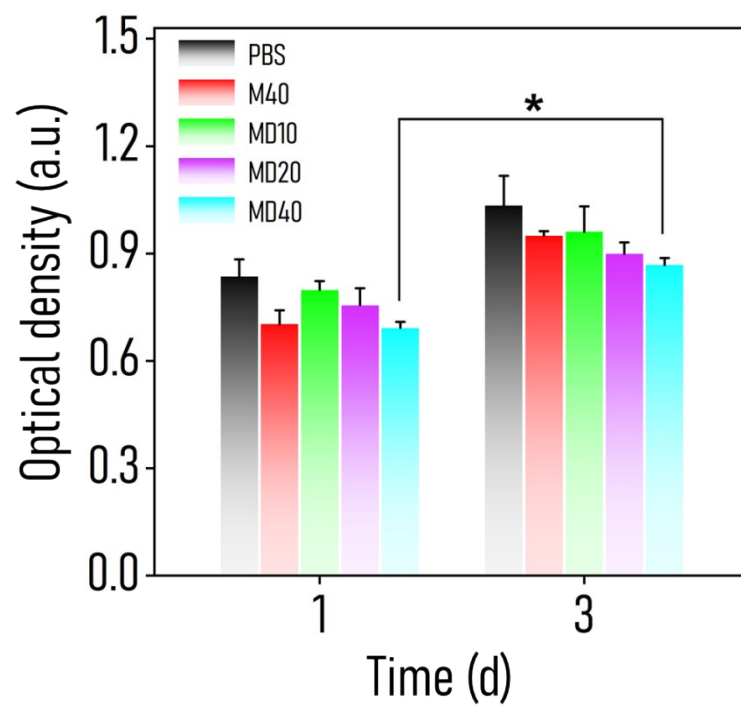


Figure S10. CCK-8 test of HUEVC cells coculturing with samples for different times. The optical density of CCK-8 solution was measured at 450 nm. (M40: 40 $\mu\text{g}/\text{mL}$ BMOF; MD40: 40 $\mu\text{g}/\text{mL}$ BMOF-DMR).

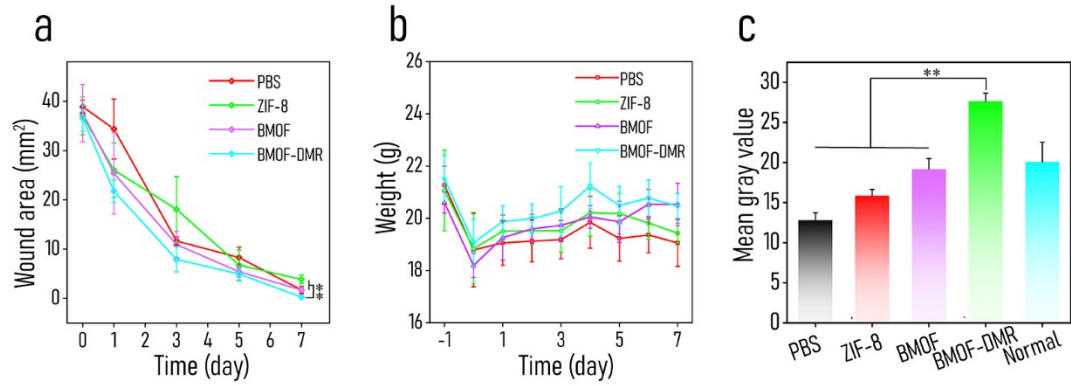


Figure S11. (a) The change curve of wound area after various treatments for different time; (b) The time-dependent weight records of mice; (c) Statistical analysis of VEGF expression by immunofluorescence in dermis.

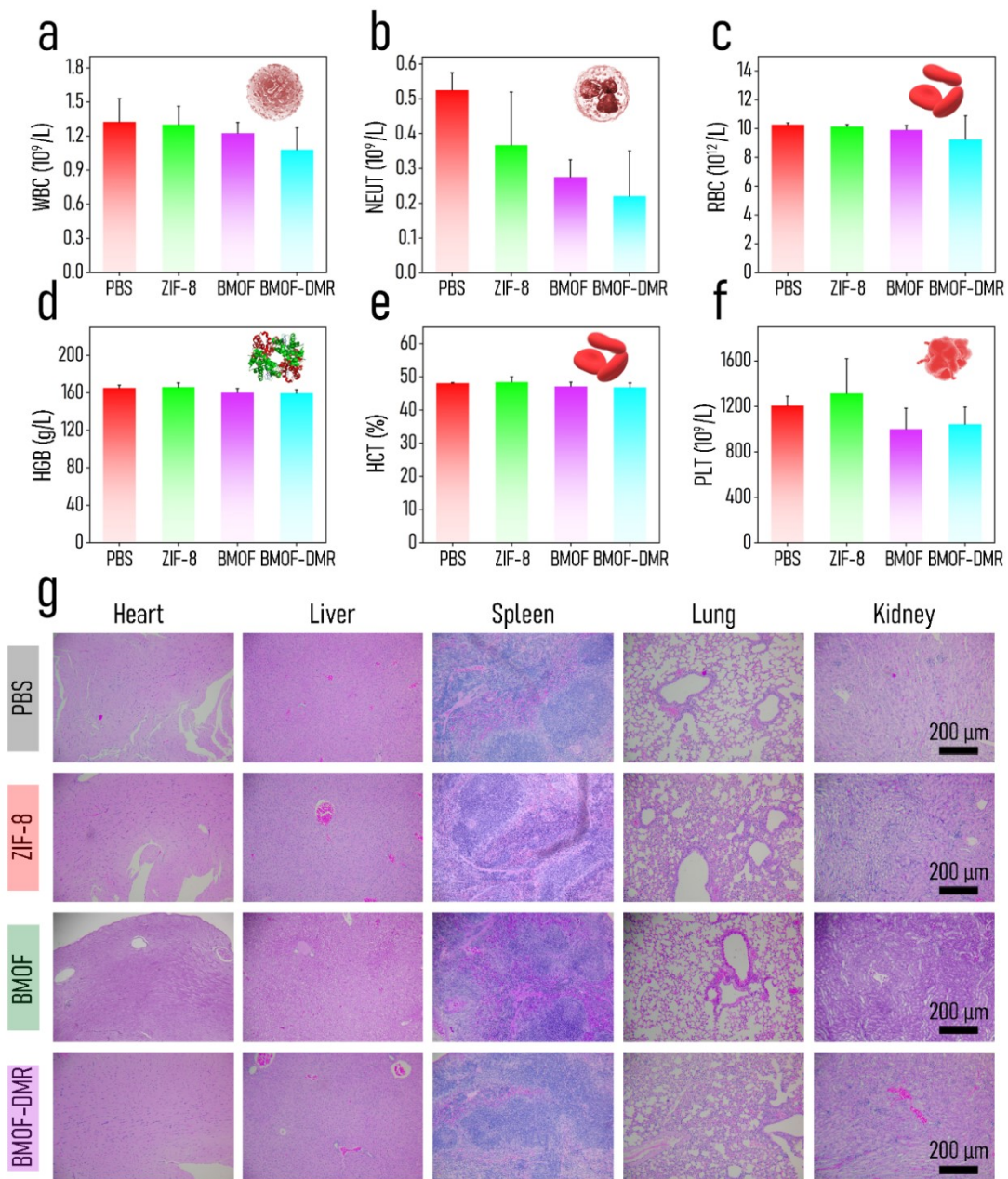


Figure S12. *In vivo* biosafety of samples after 7-d post operation of infectious wound therapy: (a-f) Whole blood biochemistry and hematology data of mice treated with samples after 7 d. The error bars indicate mean \pm SD (n = 4). (g) H&E staining of major organs (heart, liver, spleen, lung, and kidney) of PBS, ZIF-8, BMOF, and BMOF-DMR-treated mice after treatment.

Table S1. Quantitative results of the green and red fluorescence intensity in Live/Dead staining assay of bacteria.

Green signal / red signal	PBS	M40	MD40
<i>S. aureus</i>	97.2 % / 2.8 %	41.9 % / 58.1 %	22.0 % / 78.0 %
<i>E. coli</i>	95.7 % / 4.3 %	76.8 % / 23.2 %	9.8 % / 90.2 %